

Evaluation of the effect of the quality and quantity of fluid drained due to pleural effusion on complications that may develop in intensive care unit

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ABSTRACT

Aims: Pleural drainage volume is very important for oxygenation and perfusion in patients with massive pleural effusion. However, there is still no clear data between the complications that may develop after pleural drainage and the optimal volume of fluid to be removed. The primary aim of this study was to evaluate the effect of the quality and quantity of pleural fluid drained due to pleural effusion in the intensive care unit (ICU) on the complications that may develop after drainage and to determine the optimal drainage volume to prevent complications. The secondary aim was to determine the risk factors affecting the development of complications after pleural effusion drainage.

Methods: A total of 176 patients who underwent pleural drainage for pleural effusion between April 1, 2022 and December 31, 2023 in an adult tertiary ICU were retrospectively analyzed. Demographic information, clinical follow-up information, quantity and quality of pleural effusion, laboratory values and complications were recorded and the relationship between these parameters and the amount of pleural fluid drained within 24 hours and complications were evaluated.

Results: ICU duration, ICU mortality, activated partial thromboplastin time (aPTT) and vasopressor requirement were found to be statistically significantly higher in patients with complications after pleural drainage procedure. In multivariate logistic regression analysis, female gender (odds ratio=0.455, $p=0.049$) and need for vasopressors (odds ratio=2.373, $p=0.034$) increased the risk of complications. There was no statistically significant difference between the amount of pleural fluid drained and complications. In addition, when the optimal amount of drained fluid required to prevent complications was analyzed, a cut off value could not be given.

Conclusion: In order to reduce the risk of complications that may develop after pleural drainage, we believe that paying more attention to the position during pleural drainage in patients receiving vasopressor support and performing pleural drainage with the help of ultrasound in patients whose position cannot be changed due to hemodynamic disorder will reduce the complication rate. We think that a decrease in the complication rate will be effective in terms of both cost and efficient use of ICU beds by reducing the length of ICU stay and ICU mortality. In our study, the quality and quantity of pleural fluid drained had no effect on the complications that may develop after drainage, and further studies with a larger patient population are needed to investigate this situation.

Keywords: Chest tube, complications, pleural effusion, pleural volume, pneumothorax

INTRODUCTION

Pleural effusion is a common finding in intensive care unit (ICU) patients. Drainage of pleural fluid is necessary in situations such as alleviating developing symptoms, making a diagnosis, and draining an infection site.¹ Pleural infection, such as empyema, is a high-mortality diagnosis that can be missed if pleural fluid analysis is not conducted. Thoracentesis can change the likely diagnosis of the effusion in up to 45% of patients. Although pleural drainage under ultrasound

guidance is safer, serious complications such as internal organ injury, bleeding, and even death can occur during or after the drainage.²

Major complications of thoracentesis are rare.³ The primary complications are pneumothorax, hemothorax, inappropriate catheter positioning, infection, and re-expansion pulmonary edema (REPE), with pneumothorax being the most frequently observed complication, occurring with an incidence of 0-6%.^{4,5}

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This is followed by hemothorax with an incidence of 1.6%.⁶ It is thought that REPE, a rare complication, may be associated with the formation of excessive negative pleural pressure.³

The amount of fluid drained during pleural drainage is very important for oxygenation. Roch et al,⁷ have found that an increase in the PaO₂/FiO₂ ratio occurs when the volume of pleural effusion drained is greater than 500 mL. Although consensus guidelines recommend limiting the volume of pleural fluid drained at any one time to 1.5 L as a method to reduce the risk of complications, some studies have shown reasonable safety even with larger drainage volumes reaching up to 6.5 L.^{4,8} However, studies suggesting that REPE occurs as a result of draining large volumes emphasize the need to drain less than 1.5 L.^{3,4}

There are numerous studies indicating that the risk of pneumothorax increases with drainage volumes greater than 1.5 liters. On the other hand, a recent study has shown that the risk of complications is higher after small-volume drainages and that a drainage volume of 975 ml is the optimal threshold for complications.^{3,5}

Many publications have stated that there is a need for further studies due to the lack of clear data on the optimal volume of fluid to be removed after pleural drainage and the complications that may develop. The primary aim of this study is to evaluate the quality and quantity of pleural fluid drained due to pleural effusion in the ICU, its impact on complications that may develop after drainage, and to determine the optimal drainage volume to prevent complications. The secondary aim is to identify risk factors that influence the development of complications after pleural effusion drainage.

METHODS

The study was carried out with the permission of Ankara Atatürk Sanatorium Training and Research Hospital Ethics Committee (Date: 27.03.2024, Decision No 2024-BÇEK/52.)

176 patients over the age of 18 who underwent pleural drainage due to pleural effusion were included in the study at the Adult Tertiary ICU of University of Health Sciences Ankara Atatürk Sanatorium Training and Research Hospital, between April 1, 2022, and December 31, 2023..

ICU patients over the age of 18, diagnosed with massive pleural effusion through clinical findings, physical examination, and radiological assessment, and who underwent pleural drainage were included in the study. Patients under the age of 18, those who had a pleural drainage catheter placed before ICU admission, those who had drainage of spaces other than the pleural cavity, and patients admitted to the ICU due to bleeding, pneumothorax, pleural infection, or pulmonary edema, as well as those lacking necessary data for the research, were excluded from the study. Considering the amount of pleural effusion on the PA chest roentgenogram, fluid covering more than 2/3 of the hemithorax was defined as a "massive" amount of fluid.⁹

Since a patient may undergo multiple drainage procedures and each procedure carries a risk of complications, each procedure was evaluated as a separate case. In our clinic, patients

diagnosed with pleural effusion are assessed by the department of thoracic surgery, and pleural drainage is performed by an experienced doctor from the same department when deemed necessary. After pleural drainage, regular follow-up including plain chest radiographs, vital signs, physical examinations of the patients, laboratory values, and blood gas analyses are conducted to monitor for any complications. In the literature, all complications that developed after pleural drainage, including the most commonly reported complications such as pneumothorax, hemothorax, Inappropriate catheter position, pleural infection, and REPE, were recorded.

Patients' age, gender, additional diseases, Charlson comorbidity index score (CCIS), the volume of fluid drained within 24 hours via pleural drainage, the cause of the pleural effusion, presence of complications after drainage, need for vasopressors, mechanical ventilation support, length of ICU stay, ICU mortality, one-month mortality status, characteristics of the pleural effusion fluid (whether it is exudate or transudate), and laboratory values were recorded. Laboratory values included pleural fluid protein, pleural lactate dehydrogenase (LDH), and pleural culture results from the pleural fluid sample, as well as serum total protein, serum LDH, albumin, sodium, creatinine, glomerular filtration rate (GFR), leukocytes, hemoglobin, hematocrit, platelets, Prothrombin Time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), and venous blood gas analyses sent on the day of the pleural effusion drainage. The parameters observed and the volume of fluid drained were compared with the presence of complications. Additionally, the optimal volume of fluid to be drained to prevent complications was investigated.

Statistical Analysis

Data analyses were performed by using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distribution of continuous variables was normal or not was determined by the Kolmogorov Smirnov test. Levene test was used for the evaluation of homogeneity of variances. Unless specified otherwise, continuous data were described as mean, standard deviation, median (Min- max) for skewed distributions. Categorical data were described as a number of cases (%). Statistical analysis differences in not normally distributed variables between two independent groups were compared by Mann Whitney U test. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test.

Univariate and multivariate logistic regression analyses were performed to assess the association between complication the risk factors findings. Risk factors with a p-value < 0.25 in the univariate logistic regression model were included in the multivariate logistic regression analysis. Enter model used in multivariable logistic regression. Whether every independent variables were significant on model was analyzed with Wald statistic on multivariable logistic regression. It was accepted p-value <0.05 as significant level on all statistical analysis.

RESULTS

The data of 176 patients who underwent pleural drainage due to pleural effusion in a ten-bed adult tertiary general ICU were

retrospectively analyzed. **Table 1** examined the relationship between the presence of complications developed after the pleural drainage procedure and the amount of fluid drained for each complication. However, no statistically significant difference was observed between the amount of fluid drained and the complications.

Complications		Amount of fluid drained						P
		n	Mean	Standard Deviation	Median	Min	Max	
Pleural infection	No	168	1105.36	706.96	1000.0	300	4000	0.412
	Yes	8	825.00	249.28	800.0	600	1200	
Pneumothorax	No	150	1114.00	686.89	1000.0	300	4000	0.110
	Yes	26	969.23	741.23	700.0	300	3000	
Hemothorax	No	171	1088.89	695.15	1000.0	300	4000	0.633
	Yes	5	1220.00	752.99	900.0	300	2000	
Inappropriate catheter positioning	No	169	1108.28	703.26	1000.0	300	4000	0.144
	Yes	7	714.29	247.85	800.0	400	1000	
REPE	No	174	1087.93	697.50	900.0	300	4000	0.178
	Yes	2	1500.00	.00	1500.0	1500	1500	
Presence of complication	No	129	1141.09	713.12	1000.0	300	4000	0.081
	Yes	47	959.57	630.27	800.0	300	3000	

Continuous variables are expressed as median (Min-max),Mann Whitney u Test p=Level of Significance, p<0,05
REPE: Re-expansion Pulmonary Edema

According to the ROC curve analysis conducted to determine the optimal amount of fluid to be drained to prevent complications after pleural drainage, the area under the curve (AUC) was calculated as 0.586, and no statistically significant difference was found ($p>0.05$). This indicates that no specific cut off can be provided for the optimal amount of fluid drained to prevent complications (**Table 2, Figure**).

AUC	Std. Error	p	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.586	0.048	0.082	0.492	0.680

AUC:area under the curve

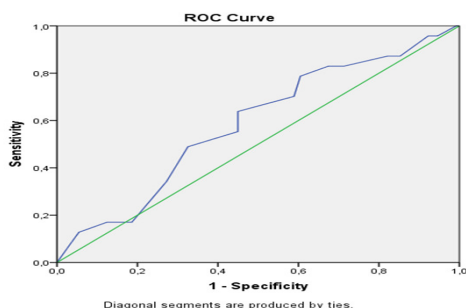


Figure. ROC curve analysis

When comparing the parameters examined in the study according to the presence of complications, the length of ICU stay and aPTT were found to be statistically significantly higher in those with complications (**Table 3**).When comparing other parameters examined in the study according to the presence of complications, the need for vasopressors and the ICU mortality

rate were found to be statistically significantly higher in those with complications (**Table 4**).

	Complication		p
	No	Yes	
Age (year)	72.00 (29.00-99.00)	70.00 (33.00-90.00)	0.495
CCIS	8.00 (0-16.00)	7.00 (0-14.00)	0.114
Length of ICU stay (day)	10.00 (2.00-110.00)	17.00 (2.00-86.00)	0.015
Leukocyte (x103/ μ l)	10.80 (0.20-66.00)	11.10 (4.60-42.00)	0.879
Hemoglobin (g/dl)	11.00 (6.60-17.10)	11.00 (6.40-16.40)	0.692
Hematocrit (%)	34.40 (20.30-72.00)	35.30 (18.60-52.00)	0.799
Platelet (x103/ μ l)	262.00 (18.00-982.00)	236.00 (54.00-558.00)	0.179
PT (s)	14.00 (10.60-59.00)	14.40 (11.40-39.00)	0.969
aPTT (s)	26.70 (12.00-113.00)	29.50 (18.90-78.00)	0.032
INR	1.20 (0.90-5.60)	1.23 (0.90-3.70)	0.965
Sodium (mmol/L)	138.00 (122.00-185.00)	138.00 (118.00-158.00)	0.968
Creatinine (mg/dl)	0.96 (0.30-5.60)	0.90 (0.36-80.00)	0.991
GFR (mL/dk/1.73 m ²)	83.00 (6.80-138.00)	72.00 (20.00-122.00)	0.894
Pleural protein (g/dl)	22.00 (11.10-49.00)	21.30 (8.70-99.00)	0.909
Total protein (g/dl)	48.00 (23.00-69.00)	50.00 (28.00-118.00)	0.941
LDH (U/L)	357.00 (146.00-987.00)	351.00 (71.00-725.00)	0.393
Pleural LDH (U/L)	165.00 (29.00-1934.00)	126.00 (35.00-15098.00)	0.159
Albumin (g/dl)	28.00 (12.50-42.00)	25.10 (17.00-40.00)	0.115
VBG pH	7.40 (6.90-7.67)	7.40 (7.03-7.65)	0.817
VBG pCO ₂ (mmHg)	42.00 (20.10-146.00)	48.00 (19.20-144.00)	0.098
VBG pO ₂ (mmHg)	41.80 (13.00-171.00)	40.00 (4.00-80.00)	0.927
VBG HCO ₃ (mmol/L)	26.00 (13.00-50.80)	27.70 (11.40-53.30)	0.249

Continuous variables are expressed as median (min-max),Mann Whitney u Test p= Level of Significance, p<0,05
CCIS:Charlson Comorbidity Index Score, ICU:Intensive Care Unit, PT: Protrombin Time, aPTT: Activated Partial Thromboplastin Time, INR: International Normalized Ratio, GFR: Glomerular Filtration Rate, LDH: Lactate Dehydrogenase, VBG: Venous blood gas, pCO₂:Partial pressure of carbon dioxide, pO₂: Partial pressure of oxygen, HCO₃: Bicarbonate

A logistic regression analysis was conducted to identify factors that increase the risk of complications among the patients included in the study. Due to the very low number of patients in individual complications, it was applied for the total number of complications. A univariate logistic regression analysis was conducted for factors thought to be associated with the risk of complications. In the univariate logistic regression analysis, the need for vasopressors (odds ratio=2.342, $p=0.018$) was considered a factor that increases the risk of complications (**Table 5**).

Variables with a p-value <0.25 from the univariate analysis were included in the multivariate analysis. The multivariate logistic regression analysis found that being female (odds ratio=0.455, $p=0.049$) and the need for vasopressors (odds ratio=2.373, $p=0.034$) were factors that increased the risk of complications (**Table 6**). Since the number of patients with individual complications was very low in our study, a comparison was made for the total number of complications. However, when we looking at which complications these two risk factors increase separately, the number of patients with pneumothorax was 10, the number of patients with pleural infection was 3, the number of patients with inappropriate catheter was 2, the number of patients with hemothorax was

2, and the number of patients with REPE was 1 in the female gender. When those who needed vasopressors were analyzed, the number of patients with pneumothorax was 12, the number of patients with inappropriate catheters was 4, the number of patients with pleural infection was 1, the number of patients with hemothorax was 1 and the number of patients with REPE was 1.

Table 4. Comparison of variables with the presence of complications-2

		Complication				
		No		Yes		
		n	%	n	%	
Gender	Female	41	31.8%	21	44.7%	0.113
	Male	88	68.2%	26	55.3%	
1-Month Mortality	No	83	64.3%	24	51.1%	0.110
	Yes	46	35.7%	23	48.9%	
Vasopressor agent Support	No	98	76.0%	27	57.4%	0.017
	Yes	31	24.0%	20	42.6%	
Need for IMV	No	64	49.6%	20	42.6%	0.407
	Yes	65	50.4%	27	57.4%	
Need for NIMV	No	99	76.7%	30	63.8%	0.087
	Yes	30	23.3%	17	36.2%	
ICU mortality	No	93	72.1%	21	44.7%	0.001
	Yes	36	27.9%	26	55.3%	
Exuda/ Transuda	Exuda	80	62.0%	23	48.9%	0.119
	Transuda	49	38.0%	24	51.1%	
Categorical variables are expressed as either frequency (percentage). Chi square Test β p=Level of Significance, p<0.05 IMV:Invasive Mechanic Ventilation, NIMV: Non-Invasive Mechanic Ventilation, ICU: Intensive Care Unit						

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Table 6. Multivariate logistic regression analysis applied to determine factors affecting complications

		Multivariate Logistic Regression			
		Wald	p	OR	95% C.I.for EXP(B)
					Lower Upper
Gender (ref kat:female)		3.871	0.049	0.455	0.208 0.997
Amount of fluid drained		1.942	0.163	1.000	0.999 1.000
Exuda/ Transuda		0.779	0.378	1.431	0.645 3.173
Vasopressor agent support		4.488	0.034	2.373	1.067 5.279
NIMV		0.099	0.753	1.160	0.460 2.926
Length of ICU stay		0.757	0.384	1.009	0.988 1.031
CCIS		2.947	0.086	0.899	0.796 1.015
Platelet		1.680	0.195	0.998	0.995 1.001
aPTT		0.657	0.418	1.012	0.983 1.042
Serum LDH		1.399	0.237	0.998	0.996 1.001
Serum albumin		2.609	0.106	0.945	0.881 1.012
VBG pH		0.212	0.645	5.791	0.003 10191.444
VBG pCO ₂		0.995	0.318	1.023	0.978 1.071
VBG HCO ₃		0.033	0.856	0.991	0.900 1.091

Wald: test statistics, OR: odds ratio, CI: Confidence interval, hosmer-lemeshow: $p>0.05$, p =Level of Significance, $p<0.05$. Statistically significant p-values are in bold.
NIMV: Non-Invasive Mechanic Ventilation, ICU: Intensive Care Unit, CCIS:Charlson Comorbidity Index Score, aPTT:Activated Partial Thromboplastin Time, LDH: Lactate Dehydrogenase, VBG:venous blood gas, pCO₂: partial pressure of carbon dioxide, HCO₃: Bicarbonate

Table 5. Univariate logistic regression analysis applied to determine factors affecting complications

		Univariate Logistic Regression			
		Wald	p	OR	95% C.I.for EXP(B)
					Lower Upper
Age		0.532	0.466	0.991	0.969 1.015
Gender		2.485	0.115	0.577	0.291 1.143
Amount of fluid drained		2.305	0.129	1.000	0.999 1.000
Exuda/ Transuda		2.404	0.121	1.704	0.869 3.341
Vasopressor agent support		5.591	0.018	2.342	1.157 4.741
Need for IMV		0.686	0.407	1.329	0.678 2.606
Need for NIMV		2.890	0.089	1.870	0.909 3.848
Length of ICU stay		2.612	0.106	1.014	0.997 1.032
CCIS		2.898	0.089	0.919	0.833 1.013
Leukocyte		0.106	0.745	1.006	0.971 1.042
Hemoglobin		0.189	0.664	0.968	0.838 1.119
Hematocrit		0.237	0.626	0.989	0.946 1.034
Platelet		2.561	0.110	0.998	0.995 1.000
PT		0.311	0.577	0.981	0.918 1.049
aPTT		1.751	0.186	1.016	0.992 1.041
INR		0.163	0.687	0.866	0.432 1.738
Sodium		0.004	0.948	0.999	0.956 1.043
Creatinine		0.909	0.340	1.046	0.953 1.148
GFR		0.006	0.941	1.000	0.989 1.010
Pleural protein		0.145	0.703	1.006	0.975 1.038
Serum Total protein		0.216	0.642	1.006	0.980 1.033
Serum LDH		1.648	0.199	0.999	0.996 1.001
Pleural LDH		1.001	0.317	1.000	1.000 1.001
Serum albumin		1.832	0.176	0.959	0.903 1.019
VBG pH		0.116	0.733	0.607	0.034 10.713
VBG pCO ₂		3.439	0.064	1.017	0.999 1.034
VBG pO ₂		0.079	0.779	0.997	0.978 1.017
VBG HCO ₃		1.701	0.192	1.027	0.987 1.069

Wald: Test statistics, OR: Odds ratio, CI: Confidence interval, hosmer-lemeshow: $p>0.05$, p =Level of significance, $p<0.05$. Statistically significant p-values are in bold.
IMV:Invasive mechanic ventilation, NIMV: Non-invasive mechanic ventilation, ICU: Intensive care unit, CCIS:Charlson comorbidity index score, PT: Protrombin time, aPTT: Activated Partial thromboplastin time, INR: International normalized ratio, GFR: Glomerular filtration rate, LDH: Lactate dehydrogenase, VBG:venous blood gas, pCO₂: Partial pressure of carbon dioxide, pO₂: Partial pressure of oxygen, HCO₃: Bicarbonate

ICU mortality rates. However, no statistically significant difference was observed between the amount of pleural fluid drained and the occurrence of complications, and no cut off value could be determined for the optimal amount of fluid to be drained to prevent complications.

Many studies have shown that spontaneous pneumothorax occurs more frequently in females.¹⁰ A study involving patients who underwent pleural effusion drainage also found that the risk of complications was higher in female patients compared to males.⁵ In our study as well, female gender was identified as a factor increasing the risk of complications after pleural drainage. This may be attributed to the lower body mass index (BMI) in females and the fact that a lower BMI is a risk factor for complications such as pneumothorax.¹¹

Patients receiving vasopressor support with hemodynamic instability often have restricted mobility. Due to the potential hemodynamic side effects of further horizontal positioning, ultrasound-guided pleural drainage is recommended in these patients.¹² In a study conducted by Park et al.,¹³ high mortality was found in patients with severe illness requiring

DISCUSSION

In this study of 176 patients who underwent pleural drainage for pleural effusion in ICU, it was found that being female and the need for vasopressors increased the risk of complications after pleural drainage. Additionally, the presence of complications was associated with higher aPTT, a longer ICU stay, and higher

vasopressors during pleural drainage, and vasopressor use was associated with mortality. In this study as well, a higher complication rate was observed in patients receiving vasopressor support during the pleural drainage procedure. This situation is attributed to the inability to adequately position patients with hemodynamic disturbances receiving vasopressor support and the lack of ultrasound use during pleural drainage procedures in our hospital.

Generally, the use of anticoagulant or antiplatelet medications, high INR, high aPTT, and low platelet count are considered the best indicators of bleeding complications during thoracentesis.³ The only absolute contraindication to thoracentesis is patient refusal, while a bleeding diathesis is a relative contraindication. Emergency thoracentesis should not be postponed even in the presence of a potential bleeding risk, depending on the risk-benefit ratio.¹³ In our study, the aPTT value was found to be statistically significantly higher in those who experienced complications after pleural drainage.

Although thoracentesis is considered low risk, complications such as pneumothorax, bleeding, and REPE are known to increase morbidity, mortality, and healthcare costs.¹⁵ In a study conducted by Bateman et al.,¹⁴ the mortality rate of 1503 patients who underwent pleural fluid drainage was found to be 43.9%, and it was observed that in-patient, 1-month, 6-month, and 1-year mortality rates, as well as hospital and ICU stay durations, were higher in patients who underwent drainage compared to those who did not.¹⁶ In another study involving 1092 patients, it was determined that complications developed after the placement of a chest tube led to an increase in hospital stay duration.¹⁷ In our study, an increase in ICU duration and ICU mortality was also observed in those who developed complications after pleural drainage.

Pleural fluid drainage can improve the ventilation-perfusion ratio by allowing the collapsed lung parenchyma to re-expand.¹⁸ Studies have suggested that the larger the volume of pleural effusion drained, the better the improvement in lung volume, respiratory mechanics, and oxygenation of the patient.^{19,20} However, the likelihood of developing REPE increases as the volume of drained pleural fluid increases.²¹ Therefore, there is no clear data on the safest volume of fluid to drain.^{3,22} The literature continues to debate the preferred method of drainage and the optimal volume of fluid to be removed.⁴ In our study, no statistically significant difference was observed between the amount of pleural fluid drained and complications. Furthermore, when investigating the optimal amount of fluid to be drained to prevent complications, no cut off value could be provided.

Limitations

Our study has some limitations. Due to its retrospective and single-center nature, the low number of patients resulted in comparing the total complication count instead of individually comparing parameters with evolving complications. Additionally, the lack of ultrasound usage during thoracentesis in our hospital prevented assessing whether there was a decrease in complications associated with ultrasound use.

CONCLUSION

Our study found that the incidence of complications following pleural effusion drainage was higher in female patients and those receiving vasopressor support. Furthermore, among those experiencing complications, higher aPTT values and higher ICU mortality rates, along with longer ICU stays, were observed compared to those without complications. To reduce the risk of complications, we believe that paying closer attention to patient positioning during pleural drainage in patients receiving vasopressor support, performing pleural drainage with ultrasound guidance in patients unable to change position due to hemodynamic instability, and prioritizing urgent pleural drainage in patients with elevated aPTT values will decrease the complication rate. We anticipate that these measures will not only reduce ICU length of stay and ICU mortality but also prove effective in terms of cost savings and efficient utilization of ICU beds. Our study did not find an association between the quality and quantity of pleural fluid drained in the ICU and the development of post-drainage complications. Therefore, further research with a larger patient population is needed to investigate this aspect.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Ankara Atatürk Sanatorium Training and Research Hospital Ethics Committee (Date: 27.03.2024 and Decision No 2024-BÇEK/52.)

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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